MARINE ACUTE TOXICITY TEST PROCEDURE AND PROTOCOL

I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable acute toxicity tests in accordance with the appropriate test protocols described below:

- **Mysid Shrimp (Mysidopsis bahia) definitive 48-hour test.**
- **Inland Silverside (Menidia beryllina) definitive 48-hour test.**

Acute toxicity data shall be reported as outlined in Section VIII.

II. METHODS

Methods to follow are those recommended by EPA in:

Weber, C.I. et al. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms, Fourth Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, August 1993, EPA/600/4-90/027F.

Any exceptions are stated herein.

III. SAMPLE COLLECTION

A discharge sample shall be collected. Aliquots shall be split from the sample, containerized and preserved (as per
40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if detected) in the laboratory using sodium thiosulfate for subsequent toxicity testing. (Note that EPA approved test methods require that samples collected for metals analyses be preserved immediately after collection.) Grab samples must be used for pH, temperature, and total residual oxidants (as per 40 CFR Part 122.21).

Standard Methods for the Examination of Water and Wastewater describes dechlorination of samples (APHA, 1992). Dechlorination can be achieved using a ratio of 6.7 mg/L anhydrous sodium thiosulfate to reduce 1.0 mg/L chlorine. A thiosulfate control (maximum amount of thiosulfate in lab control or receiving water) should also be run.

All samples held overnight shall be refrigerated at 4°C.

IV. DILUTION WATER

A grab sample of dilution water used for acute toxicity testing shall be collected at a point away from the discharge which is free from toxicity or other sources of contamination. Avoid collecting near areas of obvious road or agricultural runoff, storm sewers or other point source discharges. An additional control (0% effluent) of a standard laboratory water of known quality shall also be tested.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate standard dilution water of known quality with a conductivity, salinity, total suspended solids, and pH similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. Written requests for use of an alternative dilution water should be mailed with supporting documentation to the following address:

Director
Office of Ecosystem Protection
U.S. Environmental Protection Agency - New England
One Congress Street
Suite 1100 (Mail Code: CAA)
Boston, Massachusetts 02114-2023

It may prove beneficial to have the proposed dilution water source screened for suitability prior to toxicity testing. EPA strongly urges that screening be done prior to set up of a full definitive toxicity test any time there is question about the dilution water's ability to support acceptable performance as outlined in the 'test acceptability' section of the protocol.

V. TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA

EPA New England requires tests be performed using four replicates of each control and effluent concentration because the non-parametric statistical tests cannot be used with data from fewer replicates. The following tables summarize the accepted Mysid and Menidia toxicity test conditions and test acceptability criteria:

EPA NEW ENGLAND RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR THE MYSID, MYSIDOPSIS BAHIA 48 HOUR TEST[1]

1. Test type	Static, non-renewal
2. Salinity	25ppt ± 10 percent for all dilutions by adding dry ocean salts
3. Temperature (°C)	20°C ± 1°C or 25°C ± 1°C
4. Light quality	Ambient laboratory illumination

5. Photoperiod	16 hour light, 8 hour dark
6. Test chamber size	250 ml
7. Test solution volume	200 ml
8. Age of test organisms	1-5 days
9. No. Mysids per test chamber	10
10. No. of replicate test chambers per treatment	4
11. Total no. Mysids per test concentration	40
12. Feeding regime	Light feeding using concentrated Artemia nauplii while holding prior to initiating the test
13. Aeration[2]	None
14. Dilution water	Natural seawater, or deionized water mixed with artificial sea salts
15. Dilution factor	$\geq$ 0.5
16. Number of dilutions[3]	5 plus a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.
17. Effect measured	Mortality - no movement of body appendages on gentle prodding
18. Test acceptability	90% or greater survival of test organisms in control solution
19. Sampling requirements	For on-site tests, samples are used within 24 hours of the time that they are removed from the sampling device. For off-site tests, samples must be first used within 36 hours of collection.
20. Sample volume required	Minimum 1 liter for effluents and 2 liters for receiving waters

Footnotes:

1. Adapted from EPA/600/4-90/027F.

2. If dissolved oxygen falls below 4.0 mg/L, aerate at rate of less than 100 bubbles/min. Routine D.O. checks are recommended.

3. When receiving water is used for dilution, an additional control made up of standard laboratory dilution water (0% effluent) is required.

EPA NEW ENGLAND RECOMMENDED TOXICITY TEST CONDITIONS FOR THE INLAND SILVERSIDE, MENIDIA BERYLLINA 48 HOUR TEST[1]

1.	Test type	Static, non-renewal
2.	Salinity	25 ppt ± 2 ppt by adding dry ocean salts
3.	Temperature	20°C ± 1°C or 25°C ± 1°C
4.	Light quality	Ambient laboratory illumination
5.	Photoperiod	16 hr light, 8 hr dark
6.	Size of test vessel	250 mL (minimum)
7.	Volume of test solution	200 mL/replicate (minimum)
8.	Age of fish	9-14 days; 24 hr age range
9.	No. fish per chamber	10 (not to exceed loading limits)
10.	No. of replicate test vessels per treatment	4
11.	Total no. organisms per concentration	40
12.	Feeding regime	Light feeding using concentrated Artemia nauplii while holding prior to initiating the test

13.	Aeration[2]	None
14.	Dilution water	Natural seawater, or deionized water mixed with artificial sea salts.
15.	Dilution factor	$\geq$ 0.5
16.	Number of dilutions[3]	5 plus a control. An additional dilution at the permitted concentration (% effluent) is required if it is not included in the dilution series.
17.	Effect measured	Mortality-no movement on gentle prodding.
18.	Test acceptability	90% or greater survival of test organisms in control solution.
19.	Sampling requirements	For on-site tests, samples must be used within 24 hours of the time they are removed from the sampling device. Off-site test samples must be used within 36 hours of collection.
20.	Sample volume required	Minimum 1 liter for effluents and 2 liters for receiving waters.

Footnotes:

1. Adapted from EPA/600/4-90/027F.

2. If dissolved oxygen falls below 4.0 mg/L, aerate at rate of less than 100 bubbles/min. Routine D.O. checks recommended.

3. When receiving water is used for dilution, an additional control made up of standard laboratory dilution water (0% effluent) is required.

VI. CHEMICAL ANALYSIS

At the beginning of the static acute test, pH, salinity, and temperature must be measured at the beginning and end of each 24-hour period in each dilution and in the controls. The following chemical analyses shall be performed for each sampling event.

Minimum

Parameter	Effluent	Diluent	Quanti-fication Level (mg/L)
pH	x	x	---
Salinity	x	x	PPT(o/oo)
Total Residual Oxidants[*1]	x	x	0.05
Total Solids and Suspended Solids	x	x	---
Ammonia	x	x	0.1
Total Organic Carbon	x	x	0.5
Total Metals			
Cd	x		0.001
Cr	x		0.005
Pb	x		0.005
Cu	x		0.0025
Zn	x		0.0025
Ni	x		0.004
Al	x		0.02

Superscript:

[*1] Total Residual Oxidants

Either of the following methods from APHA (1992), Standard Methods for the Examination of Water and Wastewater, 18th or subsequent Edition(s) as approved in 40 CFR Part 136 must be used for these analyses:

-Method 4500-Cl E. Low-Level Amperometric Titration (the preferred method);

-Method 4500-Cl G. DPD Colorimetric Method, or use U.S. EPA Manual of Methods Analysis of Water or Wastes Method 330.5.

VII. TOXICITY TEST DATA ANALYSIS

LC50 Median Lethal Concentration

An estimate of the concentration of effluent or toxicant that is lethal to 50% of the test organisms during the time prescribed by the test method.

Methods of Estimation:

- Probit Method
- Spearman-Karber

- Trimmed Spearman-Karber
- Graphical

See flow chart in Figure 6 on page 77 of EPA 600/4-90/027F for appropriate method to use on a given data set.

No Observed Acute Effect Level (NOAEL)

See flow chart in Figure 13 on page 94 of EPA 600/4-90/027F.

VIII. TOXICITY TEST REPORTING

The following must be reported:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis on chain-of-custody; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended. Reference toxicity test data must be included.
- Raw data and bench sheets.
- All chemical/physical data generated. (Include minimum detection levels and minimum quantification levels.)
- Provide a description of dechlorination procedures (as applicable).
- Any other observations or test conditions affecting test outcome.
- Statistical tests used to calculate endpoints.